

Trapline foraging by bumble bees: IV. Optimization of route geometry in the absence of competition

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Foraging on resources that are fixed in space but that replenish over time, such as floral nectar and pollen, presents animals with the problem of selecting a foraging route. What can flower visitors such as bees do to optimize their foraging routes, that is, reduce return time or route distance? Some repeatedly visit a set of plants in a significantly predictable sequence (so-called “trapline foraging”), which may also enhance their foraging efficiency. A moderate level of optimization and repetition of foraging routes can be reached by following simple movement rules for choosing the distances and turning angles of successive flights, without the use of spatial memory. If pollinators can learn the locations of patches and choose among possible foraging routes or paths, however, even better performance may be achieved. We tested whether and how bumble bees can optimize and repeat their foraging routes in laboratory experiments with artificial flowers that secreted nectar at a constant rate. With increasing experience, foraging routes of bees became more repeatable and efficient than expected from a combination of simple movement rules between successive flowers. We suggest that trapline foraging is a more sophisticated pattern of spatial use than searching and is based on memory. On the other hand, certain spatial configurations of flowers hampered optimization by the bees; bees preferred to choose short distances over straight moves and showed little plasticity in this regard. Developing an efficient trapline, therefore, may require prior selection of a set of plants with an appropriate spatial configuration. *Key words:* *Bombus*, Possingham, renewing resource, spatial memory, trapline foraging, traveling salesman. [*Behav Ecol* 18:1–11 (2007)]

Because most animals must move to locate and capture food, patterns of movement or “spatial-use strategies” of foragers are considered key factors in their fitness. Spatial-use strategy is especially interesting when foragers are pollinators because it affects pollen flow or plant reproduction directly (reviewed by Handel 1983; Waddington 1983). For this reason, both pollination biologists and behavioral ecologists have studied how pollinators use space (Levin et al. 1971; Pyke 1978, 1981; Zimmerman 1979, 1981, 1982; Thomson et al. 1982; Lemke 1984; Ott et al. 1985; Zimmerman and Cook 1985; Schmid-Hempel 1986; Kipp 1987; Garber 1988; Morris 1993; Cresswell et al. 1995; Keasar et al. 1996; Cartar and Real 1997; Cresswell 1997, 2000).

Previous researchers have often assumed, implicitly or explicitly, that pollinators are foraging on flowers or plants without prior knowledge of the locations and values of rewards, as if they are “searching” in novel habitats. Hence, these studies have tended to model foraging patterns as the outcomes of simple movement rules between successively visited flowers or plants, such as choices of “movement distance” and “turning angle” (Pyke 1978, 1981; Zimmerman 1979, 1981, 1982; Waddington 1980, 1983; Schmid-Hempel 1986; Kipp 1987; Cartar and Real 1997; Cresswell 2000). This approach may not be sufficient to describe spatial use by pollinators. Bees, for example, sometimes establish small foraging areas to which they return faithfully over many days (Ribbands 1949; Singh 1950; Manning 1956; Heinrich 1976; Thomson et al.

1982; Waddington 1983; Thomson 1996; Comba 1999; Makino and Sakai 2004). Also, some nectar-feeding birds, bats, and solitary bees develop their own territories for foraging (Linhart 1973; Frankie and Baker 1974; Gill and Wolf 1975; Paton and Carpenter 1984). In foraging areas that have become familiar, how should individual pollinators use space?

Pollinators forage on gradually renewing resources such as nectar and pollen, and the locations of resource patches remain fixed. The geometry of foraging routes therefore affects efficiency in 2 ways. Depending on the speed of resource renewal and the intensity of competition, it will be most effective to visit a number of patches before returning to the starting point (Williams and Thomson 1998; Ohashi and Thomson 2005). Second, circuitous routes incur travel costs. Hence, reducing the total length of the return route could save the costs of movement, even if the same patches are visited. This problem resembles the well-known “traveling salesman” problem (Anderson 1983; Gallistel and Cramer 1996; Cramer and Gallistel 1997), a problem of finding a shortest multidestination route.

Moreover, certain pollinators are not only site faithful but also repeatedly make circuits through a particular set of patches in a predictably nonrandom order, referred to as “trapline foraging” (Manning 1956; Janzen 1971; Heinrich 1976; Ackerman et al. 1982; Dressler 1982; Lemke 1984; Gill 1988; Tiebout 1991; Thomson 1996; Garrison and Gass 1999), although the repeatability of their routes has rarely been evaluated statistically (but see Thomson et al. 1997). The repeatability of the same routes may increase foraging efficiency through a reduction of variation in elapsed time between visits on each patch. Possingham (1989) showed that a reduction of variation in elapsed time between visits on renewable resource patches will have 3 advantages: a reduction of mean resource

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standing crop or mean reward crop encountered by random visitors (“defense by exploitation”; Paton and Carpenter 1984); an increase of the mean reward crop encountered when resource renews in a decelerating way; and reduction of variation in reward crop at each visit, which will be preferred by risk-averse foragers. Possingham’s analytical results have recently been extended, and mostly corroborated, by simulation models based on somewhat more realistic assumptions (Ohashi and Thomson 2005). Natural selection should therefore favor pollinators with the ability to optimize the geometry of their routes and visit patches in their routes in a fixed order. Optimization and repetition of foraging routes can be approximated by simple movement rules. If pollinators can learn the locations of patches and choose among possible foraging routes or paths, however, they may achieve even more efficient and repeatable foraging routes.

In this study, we report laboratory experiments on the patterns of spatial use by nectar-collecting bumble bees (*Bombus impatiens*) in a large flight cage. Using artificial feeders (flowers) that secreted sugar solution at a constant rate, we monitored visit sequences of solo bees foraging on these flowers arranged in a flight cage. By challenging bees with different spatial patterns of feeders, we addressed the following specific questions: 1) Does a bee develop a repeatable foraging route or trapline? 2) Does a bee adjust its return period to the same flowers? 3) Does a bee adjust the length of its return route? 4) Are bees’ abilities to optimize and repeat foraging routes constrained by spatial configurations of flowers?

METHODS

Experiments were carried out in a flight cage measuring 788 (L) × 330 (W) × 200 (H) cm, set up in a rooftop greenhouse. Temperature ranged from 26 to 28 °C. Our subjects were workers from 2 commercial colonies of *B. impatiens* Cresson (supplied by Biobest, Leamington, Ontario, Canada). All individuals in these colonies were marked within a day of emergence with numbered, colored disks (3.0 mm in diameter)

glued onto their thoraces. Colonies were maintained in nest boxes with transparent entrance tunnels fitted with gates that allowed us to control exits and entrances. Pollen was supplied *ad lib* every day, directly to the colony. Sucrose solution was dispensed by electric artificial flowers and by a training flower, as detailed below. We used 16 workers, aged between 13 and 22 (17 on average) days since eclosion.

Artificial flowers

We used 10 identical electric artificial flowers for experiments (Figure 1). A small electric clock motor, mounted at the top of a vertical box made of clear Plexiglas, turns an axle (3.0 mm in diameter) at 1/30 rpm. The turning axle winds up a thread that is clipped to one end of a flexible reservoir: a 50 cm length of flexible tubing, 3.0 mm in internal diameter, that contains 30% sucrose solution (hereafter, “nectar”). The other end of the tube terminates in a steel needle inserted into a “flower,” comprising a “nectar bucket” (a hole 5.5 mm in diameter, 7.0 mm in depth) drilled in a horizontal platform halfway up the box. As the motor pulls upward, the nectar oozes out through the needle and accumulates in the bucket at a constant rate (2.2 µl/min). A thin plastic baffle prevents the bees from getting excess nectar directly from the steel needle hole. The depth of the nectar bucket was adjusted to the tongue length of *B. impatiens* workers so that they can empty the nectar accumulated at the bottom. Each nectar bucket was topped with a U-shaped block of plastic painted in blue, so that bees can easily find and learn to extract nectar from it.

Between experiments, we used a training flower to let bees learn where to find nectar (Figure 1c). This flower was made of a plastic vial, topped with a Plexiglas flower stage with a hole (5.5 mm in diameter) and U-shaped blue plastic block. The hole on the stage was plugged with a 3-cm wick made from a cotton dental roll, the other end of which was dipped in 20% sucrose solution in the vial. Bees could extract nectar from the surface of the wick.

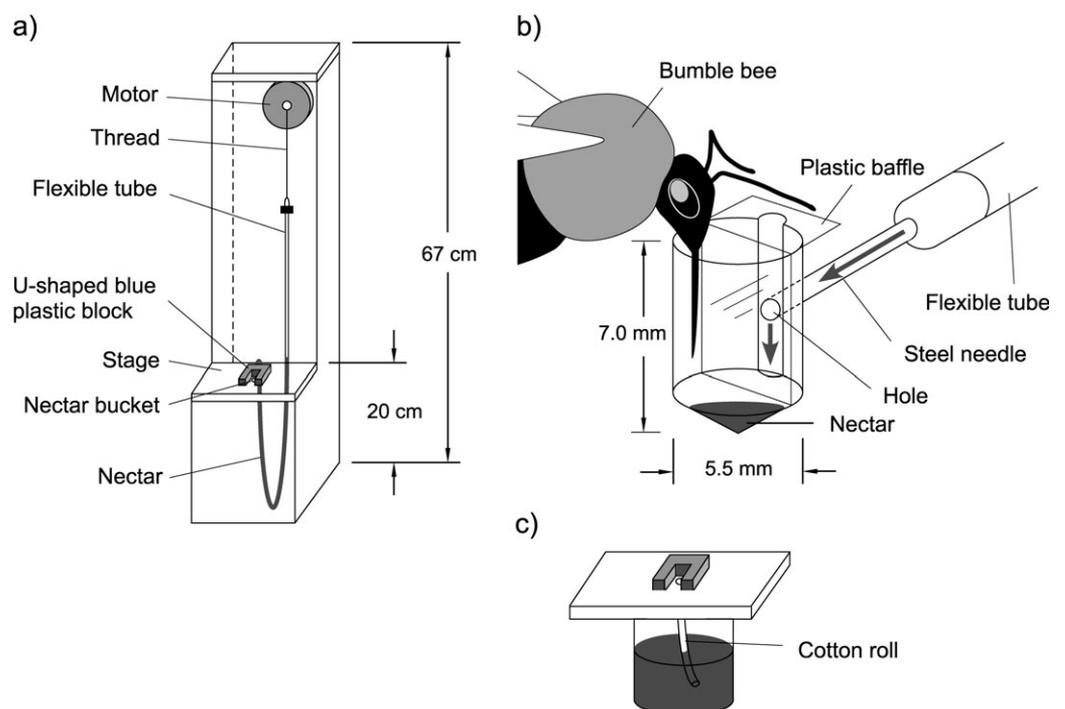


Figure 1
Views of the artificial and training flowers. (a) A whole view; (b) a close-up view of the nectar bucket; and (c) a training flower. See text for details and explanations of how each part works.

Spatial arrangements of flowers

We tested 3 configurations of floral arrays (Figure 2). These arrays displayed different relationships between 2 aspects of interflower moves: proximity and directionality. In the “independent” array, flowers always had 2–6 equidistant nearest neighbors in different directions and bees could choose movement distance and turning angle independently. In the “positive” array, proximity and directionality were positively linked, that is, the nearest neighbor (except the flower visited just before the current flower) could be reached by straight-ahead movements. In the “negative” array, on the other hand, proximity and directionality were negatively linked, that is, choosing nearest neighbors required bees to make turns.

The design of our flower, with its tower behind the flower stage, might limit bees’ departure directions even though its transparency allowed bees to see through it. To minimize such effects, therefore, we arranged all flowers so that their backs faced outside of the array where there was no other flower. In the independent array, we rotated the center flower every 10–12 trips in a clockwise direction, so that its back faced either of the 3 edges. There was no potential landmark within the array except for the flowers themselves, but there were at least 3 landmarks available near the array, including the observer, the bee nest box, and a small cage measuring 80 (L) × 50 (S) × 90 (H) cm (Figure 2).

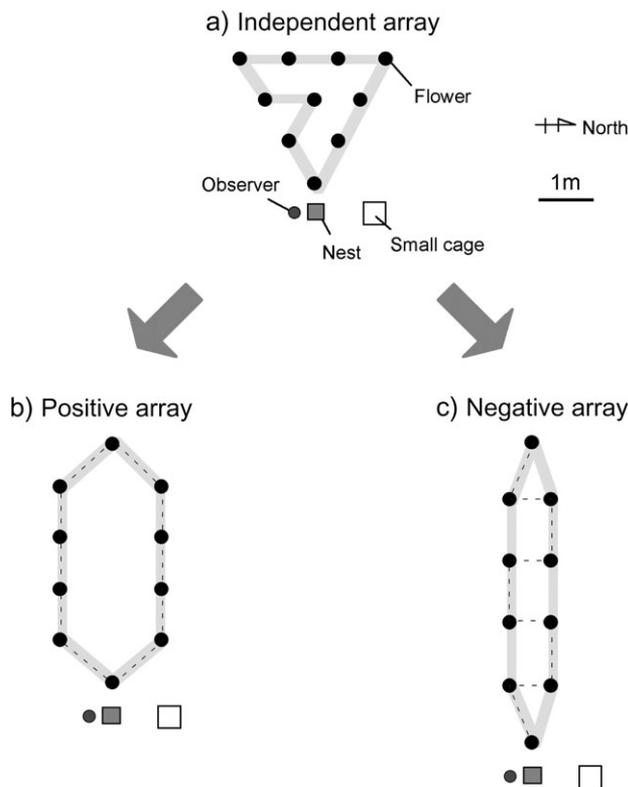


Figure 2

The 3 types of spatial arrangements of flowers used in the experiments. Closed circles indicate flowers, gray lines indicate optimal foraging routes, and dashed lines in (b) and (c) indicate routes followed by bees moving only between nearest neighbors. (a) Independent array: 10 flowers are arranged in a triangle so that bees can choose distance and turning angle independently; (b) Positive array: 10 flowers are arranged so that choices of nearest neighbors are always consistent with choices of straightest movements; and (c) Negative array: 10 flowers are arranged so that choices of nearest neighbors are often inconsistent with choices of straightest movements.

Experimental procedures

Before running an experiment, we put a colony in the cage and let bees forage freely on a training flower that was temporarily mounted on the front wall of an artificial flower. The artificial flower itself was not turned on. The location of the training flower was different from any locations of artificial flowers used in the experiments. On nonexperiment days, we left the colony open between 1000 and 1700 h. This procedure allowed bees to associate the U-shaped blue plastic block with nectar rewards, but they remained naive to any kind of spatial array of flowers.

On experiment days, we let bees forage on the training flower for 30–60 min in the morning until several bees began “regular foraging,” that is, they would visit the flower directly after entering the cage, return to the nest briefly to deposit their nectar loads, and repeat the same process. From the bees that met these criteria, we chose one for the trial. The training flower was then removed, and 10 electric artificial flowers were arranged according to one of the 3 spatial configurations. With a syringe, we removed accumulated nectar from all nectar buckets so that the first visit to each flower would not fill a bee’s honey stomach. At the beginning of the experiment, therefore, only a trace of nectar was left in each flower. Thus, the first bee visit set the nectar amount to 0, after which it accumulated linearly with time with the motors running.

We then released the focal bee only. Upon release, a bee would usually fly around in the cage but begin to forage systematically within a few minutes; if more than 15 min elapsed, we chose another bee for the experiment. After the bee visited the first 5–6 flowers, we turned on the electric motors and started nectar secretion. We sequentially numbered each flower and recorded the sequence of visits to flowers at which the bee probed for nectar (e.g., 3, 9, 4, 8). In some cases, the bee approached within 5 cm of a flower but did not land on or probe it, which was recorded as an “approach.” When the bee finished the first foraging trip and returned to the hive, we turned off the motors and waited until it reemerged. For the rest of the trial, motors were always turned on when the bee was foraging and turned off when the bee was back in the hive. The trial was continued until the bee made 60–71 (mean = 69.4) foraging trips. In this way, we observed 5 or 6 bees in each array. Each bee was tested for 5–6 h between 1000 and 1830, during March, April, and May 2003.

After each trial, we immediately placed the focal bee in a clean plastic vial and froze it at -20°C . For 12 of the 16 experimental bees, we measured the length of the radial cell on the right forewing of each bee as an estimate of body size (the other 4 bees were insufficiently preserved). We also checked the 12 bees’ hindgut contents under a microscope at $400\times$ and confirmed that none of the experimental bees had the intestinal trypanosome *Crithidia bombi* Lipa and Triggiani. *Crithidia* infections sometimes occur in commercial stocks and can affect behavior (Otterstatter et al. 2005).

Data analysis

Repeatability of foraging route

We applied several different methods to quantify the repeatability of foraging route that each bee followed. These methods were originally proposed by Thomson et al. (1997) but were slightly modified so that they fit our experimental design. Our general strategy is to calculate indices that capture certain aspects of foraging routes then examine how these indices change as a bee gains experience with a particular spatial array.

Asymmetry index. If a bee follows a particular path each time it passes through an array of flowers, the transition between an

arbitrary pair of flowers is often expected to be asymmetrical, that is, biased toward one direction. We drew a 10×10 transition matrix of interflower transitions for every 9–10 successive foraging trips made by a bee and calculated an “asymmetry index” as $-2\sum(\ln P)$, where P is a binomial probability of the observed departure from a 1:1 expectation for each pair of flowers. We omitted P values that were calculated from fewer than 6 transitions. For each bee, we obtained 7 asymmetry indices, which can be arranged in chronological order to see whether paths became more asymmetrical through time.

Skeleton diagrams. To produce graphic summaries of how often and in which direction each bee moved between 2 flowers, we calculated (as above) the binomial probability P for each pair of flowers with more than 5 transitions. On a map of flowers, we drew black arrows to indicate interflower transitions for which the bee in question showed a significant asymmetry ($P < 0.05$), with thick and thin arrows indicating frequent ($>5\%$ of all transitions) and infrequent ($\leq 5\%$ of all transitions) transitions, respectively. For a pair for which transitions were frequent but not significantly asymmetrical, we drew a white arrow with heads at both ends. To highlight movement patterns of experienced bees, we only analyzed transition data collected during the latter half of each trial.

Direct comparison of sequence data. To quantify the repeatability of visit sequences longer than pairs of flowers, we calculated a similarity index of sequences derived from a global alignment method developed for DNA sequences (Waterman 1989). The index ranges from 0 (completely dissimilar) to 1 (identical). For details and rationale, see Thomson et al. (1997). For each bee, we set the flower that was most frequently visited at the beginning of the bee’s foraging trips as the “terminal flower.” Then we scanned the data set for all sequences more than 5 flowers long that began and ended with that terminal flower. We measured the pairwise similarity between each pair of successive visit sequences picked up by this procedure. The number of similarity indices obtained for each bee varies, depending on how many pairs of successive sequences met the criteria; again, these indices can be arranged in chronological order.

Variation in return cycle. We define the “return cycle” as the number of flower visits a bee made before returning to the same flower. If a bee repeated a foraging circuit, variation among return cycles would be small. Among the 4 measurements we adopted here, this index was suggested by Thomson et al. (1997) as the best practical measure of repeatable foraging routes. We calculated the coefficient of variation (CV) of the return cycle for each trip in 2 ways: first, we considered only those cycles that fell within a single trip from the hive; second, we considered all cycles including those that spanned 2 trips. We referred to these values as the “short-term CV” and “long-term CV” of the return cycle, respectively. Whereas short-term CV will be small as long as a bee’s foraging routes are similar within each trip, long-term CV will be small only when the repeated foraging routes are also similar between successive trips. We calculated both indices for each trip unless there were fewer than 3 revisits per trip.

Average return cycle

Because each of the 10 flowers accumulated nectar linearly through time, and focal bees faced no losses to competitors, the optimal return cycle was 9. To test whether bees approximated this optimum, therefore, we calculated the average return cycle for each foraging trip.

Length of return route

To test whether bees selected the shortest route when making nine (i.e., the optimal return cycle) or more flower visits

before returning, we calculated a “traveling salesman index” (TSI), which is the ratio of the observed return route length after making more than 8 flower visits to the length of the shortest return route (i.e., the traveling salesman problem [TSP] solution), indicated as gray lines in Figure 2. To calculate TSI, we focused only on long return paths (i.e., paths including more than 8 flower visits before returning). Suppose that a bee made N long returns during one foraging trip. For each long return path, we calculated the standard return route length (SRL) as

$$\text{SRL} = (\text{Distance flown before return}) / (\text{Return cycle} + 1). \quad (1)$$

Then we calculated the average return route length (ARL) as

$$\text{ARL} = \sum \text{SRL} / N. \quad (2)$$

We also calculated the standard minimum return route length (MRL) as

$$\text{MRL} = (\text{Length of a TSP solution for the array}) / 9. \quad (3)$$

Finally, we obtained TSI for the trip as

$$\text{TSI} = \text{ARL} / \text{MRL}. \quad (4)$$

We calculated the average TSI for each foraging trip unless it contained fewer than 3 revisits with long (more than 8) return cycle.

Choice of distance and angle between successive flowers

We calculated the distances moved between flowers from the x and y coordinates of 2 successively visited flowers. We also calculated turning angles from x and y coordinates of 3 successively visited flowers as the difference between the arrival direction and the departure direction at the middle flower. Clockwise turns were taken as positive, and counterclockwise turns were taken as negative. Thus, movement angle may range from -180° to $+180^\circ$ with 0° indicating a move straight ahead.

A bee faces different sets of options for movement distances and angles, depending on the type of spatial array and the current flower on which it is feeding. To standardize these effects, we ranked each choice according to its proximity or directionality within the available options and divided the rank by the number of options. Shorter or straighter movements scored higher ranks, and \pm signs of angles were not distinguished in ranking. We referred to these measurements as “relative distance rank” and “relative angle rank,” respectively. For each foraging trip, we calculated the averages of these ranks.

Sampled randomization test with null foraging routes

Even if bees were to forage without knowledge of the locations of flowers, we would not expect them to move randomly. First, bees often prefer short and straight movements between successive flowers. When available flowers are limited, a combination of such simple movement rules may produce foraging routes with a certain level of regularity. Second, the number of flower visits during one foraging trip varied from time to time. This variation may also influence the trip-to-trip changes in efficiency and repeatability described above, even if bees did not change any other aspect of their spatial use. For example, our preliminary data analysis showed that the average return cycle decreased when bees visited only a few flowers per trip, which was often observed at the very beginning of trials. Therefore, we did not use conventional statistical approaches to test the significance of the efficiency and repeatability of bees’ foraging routes. Instead, we performed sampled randomization tests (Sokal and Rohlf 1995) to test whether the observed indices of foraging routes differed from those of null

foraging routes generated by algorithms that incorporated realistic constraints on foraging but did not include any knowledge of spatial locations.

In our algorithm, a “null bee” performs the same number of foraging trips as the observed bee. In each foraging trip, it chooses a starting flower according to the observed probability of each flower being visited at the beginning of a foraging trip. Then it continues to choose flowers sequentially according to the observed frequencies of distance and angle rank, until it has visited the same number of flowers as the observed bee did. Because the numbers of neighbors vary with a flower’s position in the array, we divided each array into 3 zones (corner, center, and edge) and calculated the zone-specific frequencies of first to ninth nearest neighbor moves from observed data. We also calculated the zone-specific frequencies of first to ninth straightest moves from observed data. Because turning angle is determined by the last 2 flowers visited, we used the position of the last flower visited before turning (corner, center, and edge) in calculating zone-specific frequencies. We used bee-specific probabilities of distance and angle ranks to test each bee’s foraging route.

A model was used to generate null foraging routes. In each step of the model, a distance rank for the next flower was drawn from the observed probability distribution. If there were only one flower with the selected distance rank, then the null bee moved to that flower. If there were two or more candidate flowers with the selected distance rank and it was the first move of the trip, one of the candidate flowers was chosen randomly for the first move of the trip. For subsequent moves, an angle rank was repeatedly drawn from observed probability distribution until it agreed with at least one of the candidate flowers. Then the null bee randomly selected its next flower from the candidates with the selected distance and angle ranks.

For each index, we obtained multiple values that could be arranged in chronological order. Using these values, we calculated a median and a Kendall’s coefficient of rank correlation between the index and the number of foraging trips experienced (or the chronological order). We also generated a set of 999 null foraging routes, with the bee’s observed movement patterns between successive flowers. We then compared the observed medians and Kendall’s taus with their null equivalents calculated from 999 null visit sequences for the bee and calculated probabilities that these values were produced by a combination of simple movement rules between successive flowers. Because our primary interest was to see whether there was a difference in a specified direction between the observed index (median or Kendall’s tau) and that from the null sequences, we calculated the one-sided probability for each randomization test. We then summarized probabilities obtained from different bees in the same array into a combined probability, using the Stouffer method for combining one-sided probabilities (Rosenthal 1991). We obtained a standard normal deviate Z for each probability P , all of which should be given as one-tailed. The Z values disagreeing in direction with the alternative hypothesis were given negative signs. Then, the sum of the Z values divided by the square root of the number of bees being combined yields a new statistic distributed as Z . If the combined probability associated with this overall Z was positive and smaller than 0.05, we rejected the null hypothesis.

RESULTS

Repeatability of foraging routes

Figure 3 shows skeleton diagrams for 16 individual bees during the latter half of the trials. By indicating the frequency and

directionality of interflower moves, each map emphasizes the portions of a bee’s movements that show repeatable structure. It is apparent that these bees frequently retraced certain pathways. Even within the same spatial configuration of flowers, however, the shapes of foraging routes differed among bees and the degree of repeatability varied among bees (diagrams are sorted from left to right by the apparent strength of repeatability).

Trends in the 4 measurements of repeatability were fairly consistent with the visual impressions derived from Figure 3; the sampled randomization tests on medians show that the repeatability of observed foraging routes was significantly higher than that of matching null visit sequences, except for the short-term CV of return cycle in the independent array (Table 1). Figure 4 shows short-term and long-term CVs of return cycle, plotted against the accumulated number of foraging trips made by the bees. In all arrays, both short-term and long-term CV decreased as the bees gained experience, although they often showed slight increases during the initial 10 trips. These trends were statistically significant, except for the short-term CV of return cycle in the negative array (Table 1). To locate the lowest level of CV, we also calculated the 10th percentiles of the observed CVs; these would be less sensitive to sample sizes than the minimum values. The 10th percentiles of the short-term CV were 0.33, 0.44, and 0.54 in the positive, independent, and negative arrays, respectively. Similarly, the 10th percentiles of the long-term CV were 0.36, 0.54, and 0.55 in the positive, independent, and negative arrays, respectively. The long-term CV was higher than the short-term CV; this difference was most evident in the independent array and least evident in the negative array. In summary, the observed foraging routes were often more repeatable than the null visit sequences. Routes tended to be more similar within trips than between trips. Moreover, repeatability of foraging routes increased as bees gained experience, but this increase was not significant for routes within trips in the negative array. The negative array, therefore, seemed to be a harder problem for bees to solve.

Number of flower visits per trip and average return cycle

In all arrays, medians of observed averages of return cycle length were significantly higher than those of null visit sequences (Table 1). In all arrays, the average return cycle increased as the bees gained experience (Figure 5). The sampled randomization tests on Kendall’s tau show, however, that this trend was not statistically significant in the negative array (Table 1). This suggests that the initial increase in the average return cycle in the negative array (Figure 5) can be explained by the increase in the number of flower visits per trip alone. To locate the highest level of average return cycle, we also calculated the 90th percentiles of the observed average return cycles, again to avoid sensitivity to small sample sizes. The 90th percentiles were 9.00, 8.00, and 8.17 in the positive, independent, and negative arrays, respectively.

The number of flower visits that a bee made during a foraging trip varied among arrays (mean \pm standard deviation of the average flower visits per trip per bee = 14.8 ± 2.2 in the positive, 23.4 ± 5.2 in the independent, and 25.9 ± 6.2 in the negative array). Significant differences were detected between the positive versus independent and positive versus negative arrays (5% significance level; Tukey–Kramer method of multiple comparisons; Sokal and Rohlf 1995). Differences among bees in the number of flower visits per trip were not significantly correlated with body size (Kendall’s tau = 0.34, $P = 0.13$, $n = 12$), and there was no significant difference among arrays in body size (Kruskal–Wallis test, $H = 3.22$, $P = 0.20$). In summary, bees returned to the same flowers at significantly

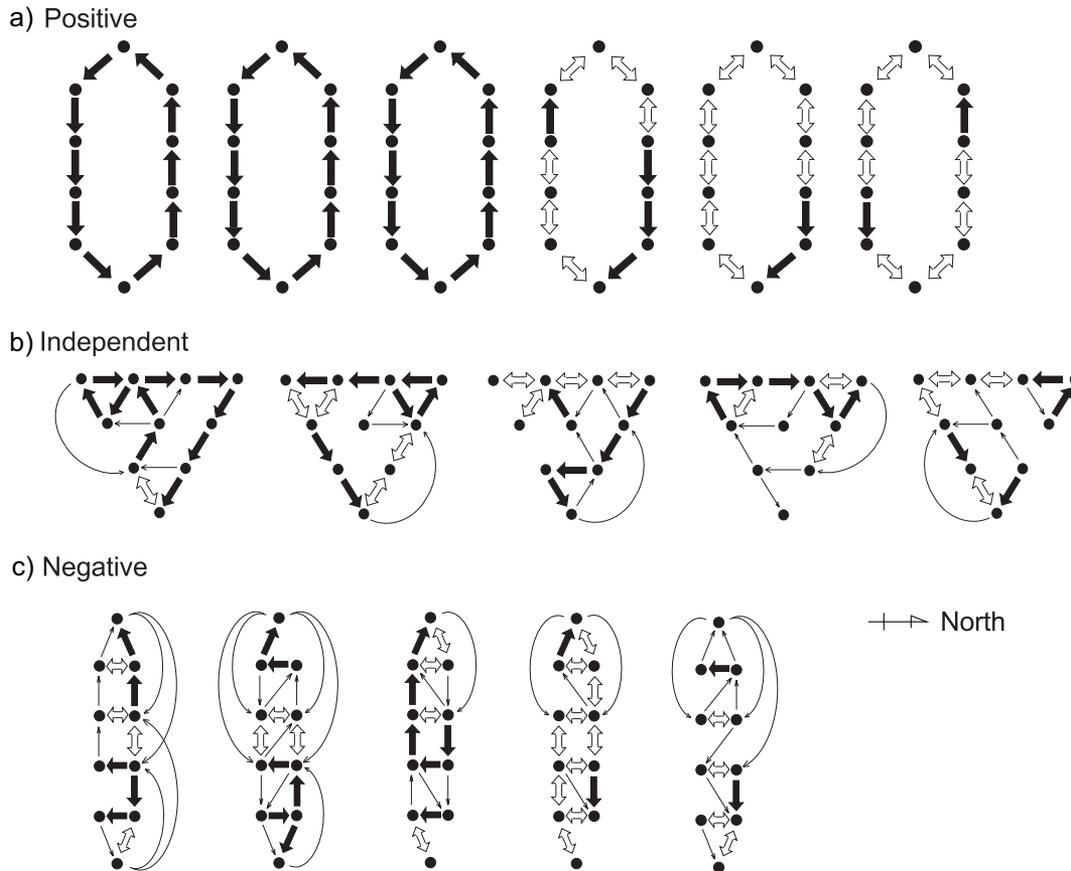


Figure 3
Skelton diagrams for individual bees. Arrows indicate how often and to which direction each bee moved between 2 flowers. Black arrows indicate significantly asymmetrical transitions ($P < 0.05$). Thick and thin arrows indicate frequent ($>5\%$ of total) and infrequent ($\leq 5\%$ of total) transitions, respectively. White arrows with their heads at both ends indicate frequent transitions without significant asymmetry.

longer intervals than those of null visit sequences. The average return cycle increased as they gained experience in the positive and independent arrays but did not change in the negative array. Experienced bees in the positive array achieved the optimal return cycle of 9 flower visits, but not those in the independent and negative arrays. This difference among arrays in the average return cycle may explain the trend in the number of flower visits per trip: because they did not return to flowers too early, bees in the positive array could have collected their fill of nectar from fewer flower visits than bees in the other arrays.

Length of return route

As shown in Table 1, bees in the positive and independent arrays followed significantly shorter routes than null visit sequences when they made more than 8 flower visits before returning. As they gained experience, moreover, bees in these arrays significantly reduced the lengths of return routes. In the negative array, however, neither trend was detected.

Choice of distance and angle in movement between flowers

Figure 6 shows the relative rank of distance and angle selected by bees in their successive flower visits. Smaller values of relative distance and angle rank indicate that bees preferred shorter and straighter movements, respectively. In the positive array, where choices of nearest neighbors were consistent

with choices of straightest movements, the relative ranks of distance and angle were equally high. In the negative array, where choices of nearest neighbors were inconsistent with choices of straightest movements, the relative rank of angle was much lower than that of distance. Bees in the independent array showed intermediate trends in choices of both distance and angle. Moreover, preferences for choosing both shorter and straighter movements increased over time in the positive and independent arrays. In the negative array, however, preference increased only for short movements but not for straight movements even when bees gained experience. Thus, bees assigned more weight to shortness than to straightness in establishing their foraging routes.

DISCUSSION

Foraging routes of bumble bees were often significantly more repeatable and efficient in geometry than expected from a combination of simple movement rules between successive flowers. Trapline foraging has been previously reported in wild bumble bees (Manning 1956; Heinrich 1976; Thomson et al. 1982, 1987; Thomson 1996; Comba 1999). In those cases, however, individual bees were allowed to choose a specific area or plants in a larger habitat. Restriction of foraging to a small number of plants will inevitably cause some repetition of interplant movements, which can give the impression that bees are systematically choosing routes even if they are not (but, for statistical approaches, see Thomson et al. 1997).

Table 1**A summary of sampled randomization tests on repeatability, efficiency, selectivity of distance and angle, and development of these aspects over time in foraging routes of *Bombus impatiens***

Index	Positive array (<i>n</i> = 6 bees)						Independent array (<i>n</i> = 5 bees)						Negative array (<i>n</i> = 5 bees)					
	Median			Kendall's tau			Median			Kendall's tau			Median			Kendall's tau		
	Mean ± SE	<i>Z</i> ^b	<i>P</i> ^c	Mean ± SE	<i>Z</i>	<i>P</i>	Mean ± SE	<i>Z</i>	<i>P</i>	Mean ± SE	<i>Z</i>	<i>P</i>	Mean ± SE	<i>Z</i>	<i>P</i>	Mean ± SE	<i>Z</i>	<i>P</i>
Repeatability																		
Asymmetry index (L) ^a	5.47 ± 1.10	5.66	7.69 × 10⁻⁹	0.27 ± 0.16	0.58	0.28	4.37 ± 0.37	7.51	2.85 × 10⁻¹⁴	0.26 ± 0.12	0.83	0.20	4.56 ± 0.54	8.03	4.44 × 10⁻¹⁶	0.17 ± 0.19	0.41	0.34
Similarity index (L)	0.44 ± 0.06	5.13	1.46 × 10⁻⁷	0.016 ± 0.08	0.37	0.35	0.21 ± 0.02	1.87	0.031	0.031 ± 0.03	0.82	0.21	0.26 ± 0.02	6.01	9.27 × 10⁻¹⁰	0.0085 ± 0.05	1.30	0.097
Short-term CV of return cycle (S)	0.61 ± 0.04	5.61	1.03 × 10⁻⁸	-0.16 ± 0.07	4.86	5.94 × 10⁻⁷	0.65 ± 0.05	-0.53	0.3	-0.048 ± 0.02	2.64	0.0042	0.75 ± 0.05	5.84	2.66 × 10⁻⁹	-0.022 ± 0.05	0.67	0.25
Long-term CV of return cycle (S)	0.66 ± 0.04	8.53	1.00 × 10⁻¹⁷	-0.23 ± 0.05	7.54	2.32 × 10⁻¹⁴	0.74 ± 0.04	4.43	4.78 × 10⁻⁶	-0.052 ± 0.05	2.36	0.009	0.75 ± 0.05	8.32	1.00 × 10⁻¹⁷	-0.041 ± 0.04	2.37	0.0089
Efficiency																		
Average return cycle (L)	6.25 ± 0.46	9.11	1.00 × 10⁻¹⁷	0.37 ± 0.09	5.64	8.34 × 10⁻⁹	6.61 ± 0.43	5.78	3.83 × 10⁻⁹	0.32 ± 0.07	3.71	0.0001	6.66 ± 0.37	8.32	1.00 × 10⁻¹⁷	-0.0023 ± 0.10	-1.33	0.091
TSI (S)	1.02 ± 0.01	6.06	6.94 × 10⁻¹⁰	-0.13 ± 0.08	2.41	0.008	1.04 ± 0.01	3.45	0.00028	-0.14 ± 0.05	3.81	6.99 × 10⁻⁵	0.98 ± 0.005	0.59	0.28	-0.064 ± 0.09	1.38	0.084
Selectivity																		
Distance (S)				-0.18 ± 0.05	6.49	4.20 × 10⁻¹¹				-0.17 ± 0.05	5.00	2.89 × 10⁻⁷				-0.76 ± 0.08	2.01	0.022
Angle (S)				-0.17 ± 0.04	5.94	1.38 × 10⁻⁹				-0.19 ± 0.07	4.84	6.62 × 10⁻⁷				-0.031 ± 0.04	0.82	0.21

^a The alternative hypothesis for the difference in index (median or Kendall's tau) between the observed and the null sequences; L, the observed index is predicted to be larger than the null; S, the observed index is predicted to be smaller than the null.

^b Each standard normal deviate *Z* was calculated from 5 to 6 bees tested in each array (see text). A positive value indicates that the overall trend agrees with the alternative hypothesis, and a negative value indicates that the overall trend disagrees with the predicted one.

^c Values of one-sided *P* are indicated in bold font when differences were significant in the direction of the alternative hypothesis.

We compared the observed medians and Kendall's taus with their null equivalents calculated from null visit sequences, produced by a combination of the observed simple movement patterns between successive flowers (see text). SE, standard error.

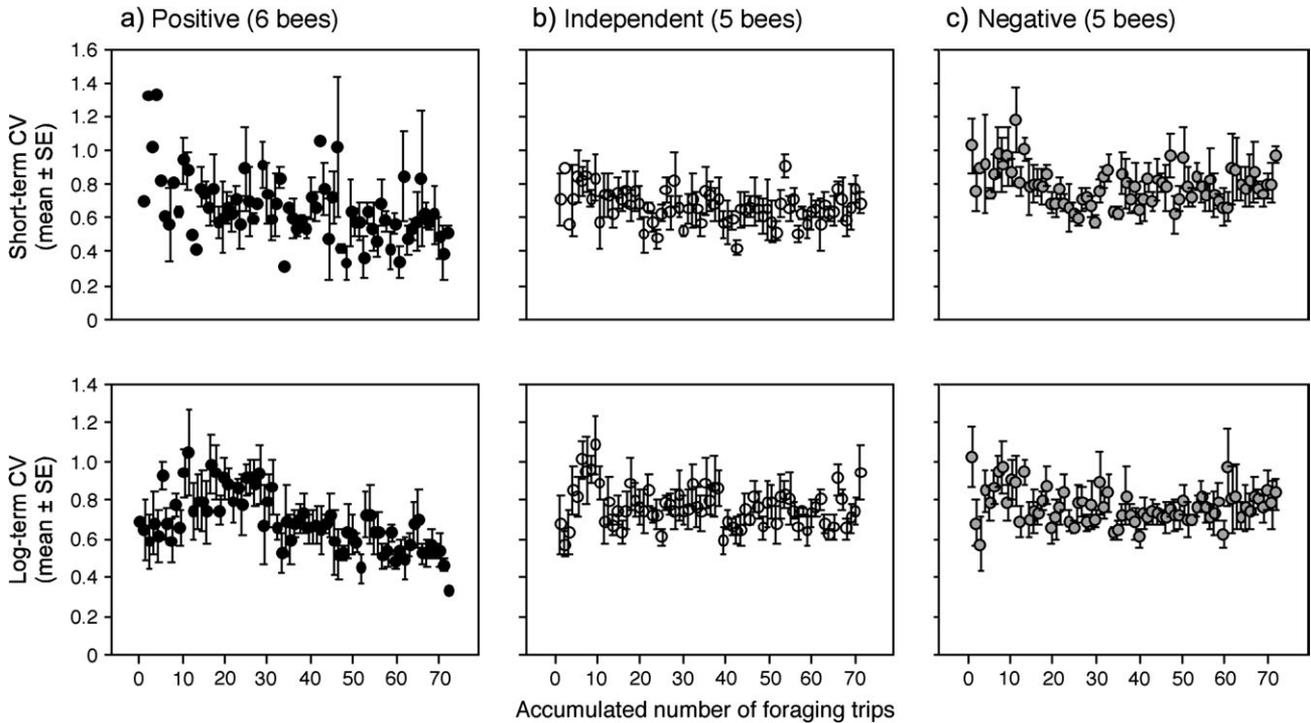


Figure 4

Changes in coefficient of variance of return cycle with accumulated foraging experience in (a) positive array, (b) independent array, and (c) negative array. Mean and standard error were calculated for each trip using data on multiple bees.

In the present study, bees were impelled to visit most flowers to collect enough nectar, by which we could demonstrate that trapline foraging was statistically detectable even when the effect of area fidelity was minimized. Moreover, the repeatability and the efficiency of foraging routes increased as naive bees gained foraging experience in an array. To date, little is known about the cognitive mechanisms that might enable such changes in geometry of foraging routes (for a review, see Collett et al. 2003). Nevertheless, our results suggest that traplining is a more sophisticated pattern of spatial use than searching driven by movement rules alone: it is based on memory of spatial locations of flowers, memory of motor patterns (Collett et al. 1993), or possibly memory of the sequential order of flowers along a route (Chameron et al. 1998).

On the other hand, we also found that the repeatability and efficiency of foraging routes varied significantly among arrays,

that is, certain spatial problems were harder for bees to solve than others. The performance differences among arrays appear to be consistent with the degree of correlation between proximity and directionality of neighboring flowers in each array, that is, the consistency of choices of nearest neighbors with choices of straightest movements. Bees best approximated the optimal foraging routes, and repeated those routes most frequently, in the positive array where the relation between proximity and directionality was positive. In contrast, foraging routes were less *repeatable* in the independent and negative arrays where the relation between proximity and directionality was independent and negative, respectively. Routes were least geometrically *efficient* in the negative array. Moreover, return periods became significantly more regular with experience in both the positive and the independent arrays, but this trend is not as clear in the negative array.

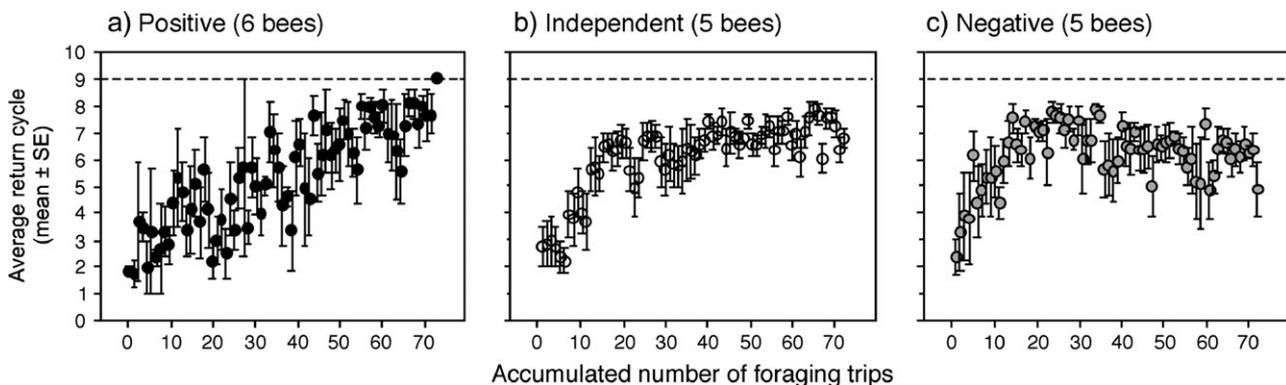


Figure 5

Changes in average return cycle with accumulated foraging experience in (a) positive array, (b) independent array, and (c) negative array. Mean and standard error were calculated for each trip using data on multiple bees. Dashed lines indicate the optimal return cycle (9 flowers).

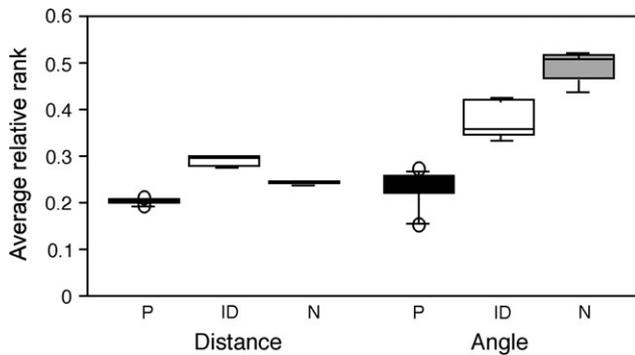


Figure 6
Patterns of choice of distance and angle between successive flowers in positive (P), independent (ID), and negative (N) arrays. Average relative distance rank and average relative angle rank were calculated for each bee, and box-whisker plots were drawn using multiple averages (P: 6 bees, ID: 5 bees, and N: 5 bees).

Similarly, route efficiency increased over time, both in the positive and independent arrays but not in the negative array.

These trends in route efficiency could be explained by relative preferences of bees for proximity and directionality in successive flower visits. When choices of nearest neighbors were consistent with choices of straightest movements (the positive array), relative distance and angle ranks were equally high. When the correlation was reversed (the negative array), the relative angle rank was much lower than the relative distance rank: when faced with conflict between choosing nearest neighbors and straightest movements, bees opted for short distances. Moreover, both the relative distance and angle ranks significantly decreased over time in the positive and the independent arrays. In the negative array, however, only the relative distance rank decreased over time. As a result, bees followed efficient circular foraging routes in the positive and the independent arrays, but they followed inefficient zigzag routes in the negative array. Their innate preference for shortness overrode their preference for straightness; their lack of plasticity in this regard limited their ability to refine their routes. Many authors have previously pointed out that bees tend to make short and straight movements (Levin and Kerster 1969; Pyke 1978; Ott et al. 1985; Kipp 1987; Cartar and Real 1997; Thomson et al. 1997; but see Zimmerman 1979, 1982). To our knowledge, however, this is the first report that bumble bees have a conservative preference to choose short distances over straight moves even in situations where that preference is not optimal. This conservative preference for short distances could be interpreted as a limitation of the bees' visual resolution (Giurfa et al. 1996) or as a decision rule to maximize short-term benefits by reducing immediate costs of movement between flowers (Stephens and Anderson 2001). It is harder to explain the observed trends in the repeatability of foraging routes. First, the weak repeatability of foraging routes in the independent array could be related to the rotational symmetry of its spatial configuration. For this array, rotations of 120 degrees always produce an identical figure (3-fold rotational symmetry), whereas the other arrays offer 2-fold symmetry (Figure 2). This higher symmetry of the independent array, together with the lack of visual landmarks within it, might have prevented bees from repeating asymmetrical foraging pathways even after they adopted asymmetrical movements between flowers (indicated by the significantly large asymmetry index in Table 1). This property may also explain, at least partly, why the long-term CV was considerably higher than the short-term CV in the independent array. Second, the lack of increased repeatability in the negative array

may be attributed to the inefficient geometry of foraging routes. Bees following zigzag routes in the negative array hit emptied flowers frequently, which might have prevented bees from experiencing continuous reinforcement and learning a particular route (e.g., Shettleworth 1998).

Bumble bees' ability to approximate the shortest return route or the TSP solution also seemed constrained by the spatial configuration of flowers. The observed average length of return route was significantly closer to the TSP solution than were the lengths of null visit sequences in the positive and independent arrays, but not in the negative array. Moreover, the TSI decreased over time in the positive and independent arrays but not significantly so in the negative array. These trends seem consistent with our results that bees' foraging routes included frequent straight paths in the positive and independent arrays, but not in the negative array. In other words, short return routes observed in the positive and independent arrays probably did not result from bees' ability to solve the TSP, but simply from their directional movements. Perhaps bees are unable to solve the traveling salesman problem because they cannot remember enough locations, although they do not necessarily have to rely on their memory of flower locations in solving the traveling salesman problem (e.g., Linhares 1998). Even if the bees can remember all the flower locations, moreover, choosing the shortest routes may require a higher level of ability to "plan ahead" or "look ahead." Cramer and Gallistel (1997) have shown that vervet monkeys choose the next segments of their foraging routes by considering at least 2 further destinations beyond the next destination (a 3-step look ahead process). However, *Bombus lapidarius* workers on a similar array of 4 flowers did not exhibit any improvement in choosing the shortest path even after 200 foraging trips (Cheverton 1982). Another possibility is that costs of following circuitous routes were relatively small in our study. The fact that bees did not always make long return paths (the observed proportion of revisits with nine or longer return cycles were $\approx 60\%$ in the positive, $\approx 30\%$ in the independent, and $\approx 40\%$ in the negative array, respectively) might have reduced the relative importance of solving TSP. Even if long return paths were frequent enough, a failure to solve TSP would cause only a slight increase in movement costs (e.g., the maximum excess distance in the independent array was 38 cm, which was only 4% increase from the TSP solution). With no competition, and with linear resource renewal as in this study, the penalty costs of circuitous routes may be trivial because an increase in return time will be counterbalanced by an increase in reward crop encountered (Ohashi and Thomson 2005).

These results suggest that a bee's ability to optimize its foraging route in nature may depend largely on how it selects a set of plants or patches in a large habitat. For a route-based forager, the profitability of any particular plant or patch is a function of its locations relative to neighboring plants, as well as of its own reward value. If it has sufficient options, a bee might select a set of plants or patches with a configuration similar to our positive array: a circular or oval arrangement of plants in which choices of nearest neighbors are consistent with choices of directional movements. It would be interesting to test whether the established traplines of bumble bees in the field meet these conditions and whether a bee's foraging route converges to a "positive arrangement" as it gains experience. In hexagonal arrays of 37 plants, both Thomson et al. (1997) and Makino and Sakai (2004) found that bees commonly performed circuits along the edges of the arrays, but a tendency to follow edges could easily be attributed to simple biases in movement rules. Moreover, it has been suggested in field experiments that bumble bees avoid intensive overlap of their foraging areas with competitors

(Thomson et al. 1987). This tendency would limit available spatial configurations of plants or patches, so that individual bees may not always use geometrically ideal sets of patches or plants as their foraging areas. Such a limitation may increase variation in foraging efficiency or repeatability among individuals under competitive situations. We plan to examine competition in further studies and hope that our present results will encourage future work on foraging area selection and the geometry of learned foraging routes for animals collecting renewing resources from isolated patches in the field.

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